

Applicants: Nathan Ellis, James German, and Joanna Groden
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Amendments to the Specification:

Please amend the paragraph on page 3, line 29 through page 4, line 18 as follows:

Figure 1A represents the genetic map of the *BLM* region of 15q. On the upper horizontal line, the order and distances (shown in kilobase "kb") between the polymorphic microsatellite loci were estimated by long-range-restriction mapping (~~Straughen, J., et al. Physical mapping of the bloom syndrome region by the identification of YAC and P1 clones from human chromosome 15 band q26.1. Genomics 35(1): 118-128, 1996).~~ The distance between *D15S127* and *FES* (not indicated) was determined to be 30 kb by restriction enzyme mapping of a cosmid contig (see below). Vertical lines indicate the position of the marker loci, and the circle represents the centromere. The interval between loci *D15S1108* and *D15S127* is expanded below the map. Vertical lines intersecting mark the unmethylated CpG-rich regions identified by long-range restriction mapping, and arrows indicate the direction of transcription of three genes in the region. Certain YACS, PIs, and cosmids (Y, P, and c, respectively) from the contig (~~Straughen, et al., supra~~) are depicted by horizontal lines underneath the map. Dashes on the YAC lines indicate internal deletions. At the top of the figure, the horizontal cross-hatched bars indicate regions proximal to *BLM* that remained heterozygous in the low-SCE LCLs and regions distal to *BLM* that had become homozygous. The minimal region to which *BLM* was thus assigned by SCP mapping is represented in black.

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Please amend the paragraph on page 4, line 19 through page 5, line 11 as follows.
Note that the underlining of "Clin. Genet." appears in the original text.

Figure 1B represents the autoradiographic evidence showing heterozygosity proximal to *BLM* and reduction to homozygosity distal to *BLM*. The four persons of five from whom low-SCE LCLs had been established that were informative at *D15S1108* or *D15S127* are shown. To determine both the constitutional and the recombinant cell line genotypes, PCRs were carried out using DNA samples prepared from high-SCE cells (P^h) and low-SCE LCLs (P^l) of persons with BS as well as samples from their fathers (PF) and their mothers (PM). These persons are identified by their Bloom's Syndrome Registry designations (see German, J., and Passarge, E. Clin. Genet. 35:57-69 (1989)). Arrows point to DNA fragments amplified from the heterozygous alleles of the constitutional genotypes, pat (for paternal) and mat (for maternal). Asterisks mark alleles in the low-SCE LCLs that are lost through somatic crossing-over. Lines mark DNA fragments amplified from alleles of the parents but that were not transmitted to the offspring with BS. From one of the four persons with BS, 11 different clonal LCLs were examined; 3 of the 11 had undergone reduction to homozygosity at loci distal to *BLM* --as explained elsewhere ((~~Ellis, N. A., et al. Somatic intragenic recombination within the mutated locus *BLM* can correct the high sister-chromatin-exchange high-SCE phenotype of Bloom syndrome cells. Am. J. Hum. Genet. 57(5): 1019-27, 1995, Erratum in Am. J. Hum. Genet. 58(1): 254, 1996).~~). Autoradiographic patterns are shown from 2 of the 11 low-SCE LCLs from 11(IaTh), one representative of cell lines in which allele losses were detected (P^l sample on right) and another of cell lines in which they were not (P^l sample on left).

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Please amend the paragraph on page 13, line 28 through page 14, line 16 as follows. Note that the underlining of "supra" and the Journal titles appear in the original text.

The persons with BS in whom low-SCE lymphocytes have arisen were described previously (~~German, J., et al. Bloom's syndrome. XIX. Cytogenetic and population evidence for genetic heterogeneity. Clin. Genet. 49(5):223-231, 1996).~~ Epstein-Barr virus transformed lymphoblastoid cell lines (LCLs) were developed from these and other persons with BS by standard culture methods using material obtained through the Bloom's Syndrome Registry (German and Passarge, supra). The recombinant low-SCE LCLs in which reduction to homozygosity had been detected, and the cells used to determine the constitutional genotypes of the five persons from whom these recombinant low-SCE LCLs were developed, also have been described (~~Ellis, et al. Am. J. Hum. Genet., 1995, supra).~~ The polymorphic loci typed included some previously reported (Beckmann, J. S., et al. Hum. Mol. Genet. 2:2019-2030 (1993); Gyappay, G., et al. Nature Genetics 7:246-339 (1994)) and others that were identified during the physical mapping of the BLM region of chromosome 15 (~~Straughen, et al., supra).~~ The methods of preparation of DNA samples, oligonucleotide primers, and conditions for PCR amplification of microsatellite polymorphisms on chromosome 15 have been described (German, et al., 1994, supra; Ellis, N. A., et al. Am. J. Hum. Genet. 55:453-460 (1994); ~~Straughen, et al., supra).~~

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Please amend the paragraph on page 19, line 23, through page 20, line 8, as follows. Note that the underlining of "supra" appear in the original text.

BLM previously was localized by SCP mapping to a 1.3 cM interval bounded proximally by *D15S116* and distally by four tightly linked loci *D15S127*, *FES*, *D15S158*, and *IP15M9* (Ellis, et al., ~~Am. J. Hum. Genet.~~, 1995, supra). The four loci are present in a 1-2 cM interval on chromosome 15 (Beckmann, et al., supra; Gyappay, et al., supra). The order of these four loci was determined by PCR analysis of clones in a 2-Mb YAC and P1 contig that encompasses *BLM* (~~Straughen, et al.~~, supra). The four loci were oriented with respect to the telomere by finding a recombinant chromosome in a BS family in which crossing-over had occurred between *BLM* and *IP15M9*, placing *IP15M9* on the distal end of the contig (Fig. 1A). Because *D15S127* was the most proximal locus that was reduced to homozygosity in low-SCE LCLs, polymorphic loci in the region proximal to it were sought. There, a polymorphic locus, *D15S1108*, was identified that remained constitutionally heterozygous in the recombinant low-SCE LCLs, in contrast to locus *D15S127* that had become homozygous in them (Fig. 1B). This shift from heterozygosity to homozygosity of markers indicated that *BLM* is situated in the 250-kb region between *D15S1108* and *D15S127*.

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Please amend the paragraph on page 24, line 22, through page 25, line 10, as follows. Note that the underlining of "supra" appear in the original text.

In the present study, *BLM* was isolated by a positional cloning strategy. *BLM* first was localized by homozygosity mapping to a 2-cM interval flanking *FES* (German, et al., 1994, supra), a gene already mapped to chromosome band 15q26.1. A 2-Mb YAC and P1 contig encompassing *FES* was constructed, and closely spaced polymorphic DNA markers in the contig were identified (~~Straughen, et al., supra~~). *BLM* then was assigned by SCP mapping to a 250-kb interval in the contig, one bounded by the polymorphic loci *D15S1108* and *D15S127* (Fig. 1). A cDNA clone (905-28) was isolated by direct cDNA selection using a cosmid clone from the interval, and cDNA analysis identified the 4,437-bp H1-5' sequence (Fig. 2). This sequence encodes a putative peptide homologous to the RecQ helicases (Fig. 4). RNA transcripts 4.5-kb long were identified by Northern blot analysis (Fig. 5A), and electrophoretic abnormalities in RNAs were detected in cells from seven unrelated persons with BS, suggesting that these RNAs are derived from mutant *BLM* genes (Fig. 5B). Finally, RT-PCR/SSCP analysis disclosed 7 unique mutations in 10 persons with BS (Table 1; Fig. 6), 4 that are chain-terminating and 3 that are putative missense substitutions, 2 of the 3 affecting amino acid residues conserved in RecQ helicases and the third changing a cysteine to a serine.

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Please amend the paragraph on page 26, lines 11-30, as follows. Note that the underlining of "supra" and the Journal titles appear in the original text.

The problem of too little positional information in available families can be mitigated in exceptional situations in which linkage disequilibrium between the disease-associated gene and tightly-linked polymorphisms can be detected in a genetic isolate. In these cases localization of a gene to a short interval in the genome by haplotype analysis can be more exact than is possible using standard linkage analysis of family data (e.g., Kerem, B.-S., et al. Science 245:1073-1080 (1989); Sirugo, G., et al. Am. J. Hum. Genet. 50:559-566 (1992); Lehesjoki, A. E., et al. Hum. Mol. Genet. 2:1229-1234 (1993); Hastbacka, J., et al. Cell 78:1073-1087 (1994)). Linkage disequilibrium in fact was a strategy available in BS (Ellis, et al., Am. J. Hum. Genet., 1994, supra), and it permitted a minimum regional assignment of BLM to the same 250-kb interval described herein (Ellis, N.A. et al., The Ashkenazic Jewish Bloom syndrome mutation blm^{Ash} is present in non-Jewish Americans of Spanish ancestry, Am. J. Hum. Genet. 63(6): 1685-93, 1998). This approach could have allowed the inventors to clone *BLM*. Instead, the inventors carried out SCP mapping first.

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Please amend the paragraph on page 26, line 31, through page 27, line 13, as follows:

In the SCP-mapping strategy, the inventors took advantage of recombinant cell lines from BS somatic cells in which crossing-over within *BLM* had taken place, resulting in the correction of the mutant phenotype in their progenies (~~Ellis, et al., Am. J. Hum. Genet., 1995, supra~~). After a segregational event, all polymorphic loci distal to *BLM* were reduced to homozygosity in half of the cases of intragenic recombination. This mapping method was preferred to linkage-disequilibrium mapping because the crossovers that permitted localization of *BLM* had occurred within the gene itself and fewer genotypes were required for the analysis. By genotyping polymorphic loci that flank *BLM* in high-SCE and low-SCE samples from only five persons with BS and their parents, the position of *BLM* was delimited to the short interval bounded by the marker loci *D15S1108* and *D15S127* (Fig. 1). With *BLM* assigned to such a short interval the cloning of *BLM* became straightforward. The first candidate gene isolated from the interval proved to be *BLM*.

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Please amend the paragraph on page 28, line 17, through page 29, line 4, as follows:

BS is an autosomal recessive with high penetrance and expressivity. The observation of loss-of-function mutations in *BLM* helps to explain these genetic characteristics. The short stature, characteristic facies, facial sun-sensitivity, hyper- and hypopigmented patches on the skin, immunodeficiency, male infertility, female subfertility, premature menopause, and the predispositions to late-onset diabetes and to neoplasia exist in virtually all groups of persons with the syndrome. The BS phenotype is similar in the Ashkenazi Jews, the Dutch, Flemish, German, Italian, Greek, Turkish, and Japanese--i.e., wherever it's been diagnosed. In addition, the elevated chromatid exchange and the hypermutability are constant cellular manifestations. No more variability in the expressivity of the mutations has been detected in persons with BS who inherit an identical mutation by descent from a common ancestor, as happens in Ashkenazi Jews with BS and in the 25% of non-Ashkenazi Jewish persons with BS whose parents are cousins, than has been detected in persons who are compound heterozygotes (~~German et al., 1995, supra~~). Nevertheless, with *BLM* cloned, it is possible to identify the mutations in any person with BS, and more subtle genotype-phenotype correlations now can be carried out.